Botany. — *On the development of the stellate form of the pith cells of Juncus species.* II. By R. A. MAAS GEESTERANUS. (Communicated by Prof. G. VAN ITERSON.)

(Communicated at the meeting of April 26, 1941.)

3. *The composition and the double refraction of the walls of the pith cells.*

The walls of the juvenile pith cells of *Juncus* are clearly stainable with ruthene red, while methylene blue gives to them a blue colour, which disappears with acetic acid. All this proves that the walls contain pectin. These walls only give a very weak cellulose reaction, while lignin reactions are entirely negative.

The walls of adult pith cells still give pectin reactions. If no preliminary treatment is given the cellulose reaction is weak in such walls, but after treatment with "eau de Javelle" it becomes strong. This is to be ascribed to the presence of lignin in those walls, though the re-agents to lignin on the adult walls do not give strong reactions and though the walls of the vascular bundles in the leaf prove to be much richer in lignin than those of the stellate cells.

When we examine with the polarization microscope the walls of very juvenile pith cells, as given for instance in fig. 5, no double refraction is seen, but it does occur when the cell arms begin to be visible, as is the case at the stage of fig. 8. Then the thickness of the cell walls is between 0.70 and 1.05 μ but at this stage the walls do not yet contain lignin.

When between crossed nicols a plate of red 1st order is inserted under 45°, placing an arm of a stellate pith cell with the longitudinal direction also under 45°, then in the plane aspect of the cell arm is seen the substraction colour: yellow 1st order. Closer study of the double refraction of the wall of the arm also shows that the shortest axis of the index ellipsoid in that wall is perpendicular to the face of the wall, as is always the case in cell walls consisting of cellulose, and that the longest axis is parallel to that face and perpendicular to the longitudinal direction of the arm.

So in this stage of development of the pith cells the cellulose micellae in the walls of the arms have a preference for a direction transverse to the longitudinal direction of the arms (it should be remembered that pectin is only very weakly double refractory, so that from the double refraction we can only draw a conclusion with reference to the cellulose cels). So the walls of these arms have a so-called "Röhrenstruktur", in the nomenclature of A. FREY WYSSLING (7).

We have considered if at this stage there is a connection between the strength of the double refraction and the length of the cell arms. This double refraction we have determined in natrium light with the aid of a compensator — according to A. EHRINGHAUS — in the walls in face aspect (it should be borne in mind that the double refraction is determined of the upper and the lower walls of the arm simultaneously). The results we found for 37 cell arms are represented graphically in a correlation table (see fig. 16), in which the lengths of the arms are given in microns and

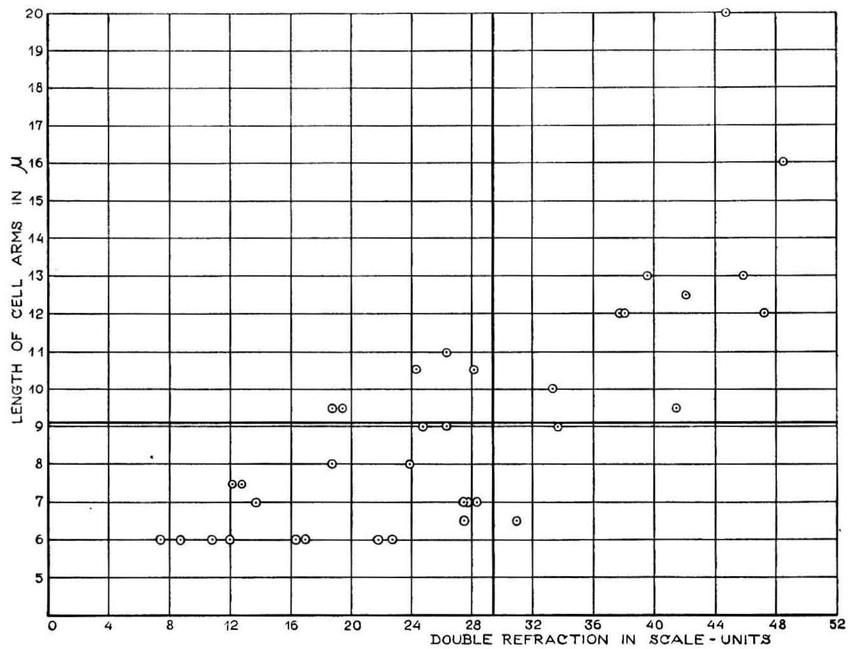

Fig. 16.

Correlation scheme for the double refraction of the (double) wall of the arms of the stellate cells of *Juncus effusus* and the length of these arms; the distribution of the values over the 4 quadrants of the scheme proves the existence of an evidently positive correlation.

the double refraction in units of the scale of the compensator. From that table it is seen that there is an evident correlation for the two quantities and that as a rule the longer cell arms also show higher double refraction.

When the cell arms of adult pith cells are studied with the polarization microscope, it is seen that in the same position in which the arms of more juvenile cells had a substraction colour, the adult cells show an addition colour. So in the layer which has now been formed against the inner side of the walls (at this stage the thickness of the wall varies between 1.40 and 1.80 μ) the cellulose micellae lie preferably in the longitudinal direction of the cell arms.

At stages of development situated between the two stages of development mentioned last, transitions may be observed a.o. cell arms may be found which, seen from above, seem to be isotropic; in this case the double refraction of the first cell wall and that of the later added layer neutralize each other.

4. *Observations about the method of development of the cell arms.*

We have seen in the introduction to this communication that the cause of the occurrence of the stellate form of the cells in the adult pith was frequently sought in the stretching of the pith by the rapidly growing surrounding tissue.

We will now first communicate an experiment which supports this conception. From a disk-like sheet of thin vulcanized rubber (we used "Traun's Ideal Rubber Dam" of Traun Rubber Co. Atlantic Rubber MFG. Corporation Successors Med. 6") we cut away a triangle as shown in fig. 17. After that we stretched the sheet uniformly in all directions by fixing the edge of the disk between two metal rings. Next the disk was pulled over the wide neck of a glass jar. The triangular opening was changed into a circular one as seen in fig. 18.

It is at once evident that the change in the shape of the opening is quite like what we observed in the pith at the transition from the stage of fig. 5 to the stages of figures 6 and 7.

We draw the reader's attention to the fact that near the vertices of the original triangle the sheet has become very transparent (the photo was taken with transmitted light); here the sheet was stretched most. This was confirmed by observation of the polarization colours, which were seen when the sheet was viewed between two large polaroid filters with a diameter of 4 cm of the "Polaroid Corporation, Boston Mass". As proved when a gypsum plate red 1st order was inserted the micellae in the rubber sheet near those vertices were placed parallel to the periphery of the round opening (perpendicular to the black lines in fig. 18). From this it follows that the direction of the micellae near the opening in the rubber sheet is different from the direction observed in the cell walls near the intercellular spaces.

Still more striking perhaps is the experiment depicted in figures 19 and 20. Fig. 19 shows a rubber sheet on which we have drawn a system of hexagons and into which we afterwards punched small circles at the vertices of these hexagons. In fig. 20 it is seen how the sheet is changed by uniform stretching. The hexagons have obtained a stellate form. Here we have as it were a demonstration of the transition from the stage shown in fig. 7 to that of fig. 9.

In agreement with our remark concerning fig. 18, the micellae in the arms of the stellate figures in fig. 20 lie in the direction of those arms and not transverse to that direction, as is the case in the arms of the pith cells of *Juncus* before the thickening process. From what we remarked about the location of the micellae it follows that an explanation which would account

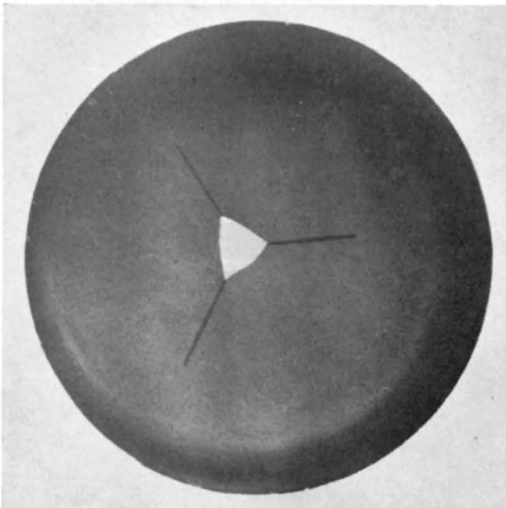

Fig. 17.
Thin sheet of vulcanized rubber, in which a triangular opening has been cut.

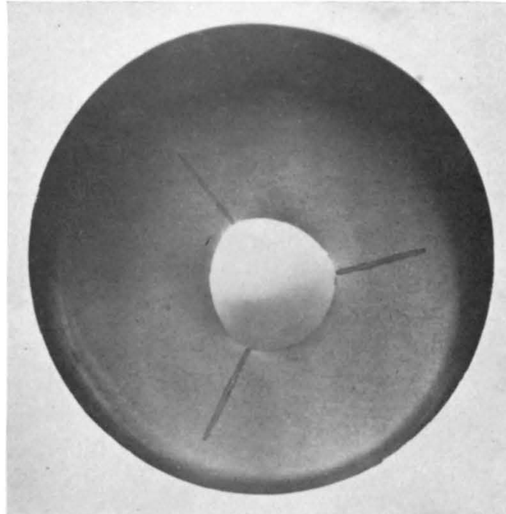

Fig. 18.
The sheet, shown in fig. 17 in non-stretched condition, is here depicted after uniform stretching in radial directions; the triangular opening has been become round; the stretching is strongest near — namely perpendicular to — the inklines.

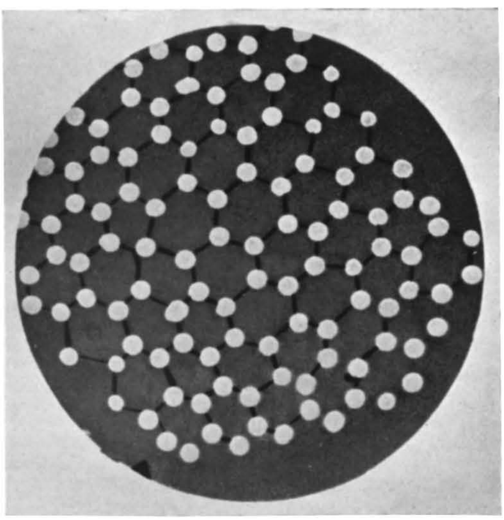

Fig. 19.
Thin sheet of vulcanized rubber, on which a system of hexagonal figures has been drawn with ink, and in which round openings have been punched in the vertices of those figures.

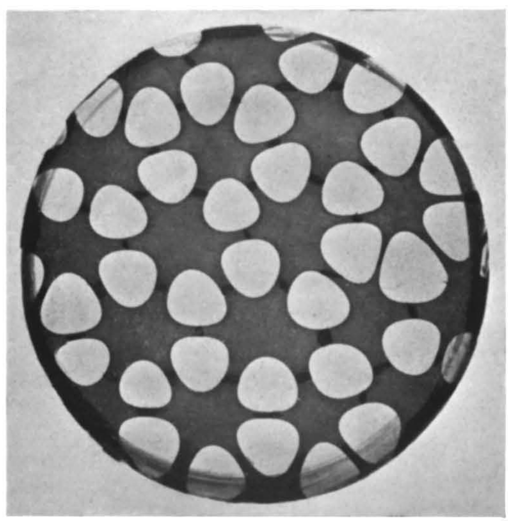

Fig. 20.
The sheet, shown in fig. 19 in non-stretched condition, is here shown after uniform stretching in radial directions; the rubber between the openings assumes the shape of the stellate cells of *Juncus effusus*; here the micellae in the arms have a preference for the longitudinal direction of those arms.

for the stellate form of the pith cells by stretching by the surrounding tissue only cannot be sufficient.

We would observe that as soon as the cell arms have been formed, they may be compared with bodies with a cylindrical wall, which are inflated by inward pressure — in this case by the turgor pressure. In 1937 G. VAN ITERSON Jr (10) drew our attention to the fact that in such a case the greatest tension in the cylindrical wall is not in the direction of the axis, but transverse to it. If the wall of such a body is elastic and can be stretched in all directions to the same extent, it will be stretched most in transverse direction. VAN ITERSON demonstrated this by inflating cylindrical rubber balloons on which squares had been drawn. Such a balloon before and after inflation is shown in fig. 39 of a treatise by Miss. J. M. DIEHL, Miss C. J. GORTER, G. VAN ITERSON Jr. and Miss. A. KLEINHOONTE (1939) (3).

Almost simultaneously with VAN ITERSON, E. C. CASTLE (1937) had arrived at the same conclusion by another method.

If one assumes with VAN ITERSON and with CASTLE that the expansion of the walls determines the direction of the (rod-shaped) cellulose micellae, then it will be clear that the micellae are placed transversely to the direction of the arms, if at least the mechanical stretching of those arms by the surrounding tissue does not neutralize this location. Apparently this is not the case and therefore in our opinion the position of the micellae in the yet unthickened arms of the stellate cells should not be ascribed to the stretching by the surrounding tissue, but to stretching through turgor pressure. Possibly the mechanical stretching of the cell arms by the surrounding tissue occurs only in the first stages, i.e. before the stage of fig. 7.

VAN ITERSON has shown that it was already known to earlier investigators that in walls of cylindrical plant cells, growing in the direction of the cylindre axis, the micellae are generally placed transversely to this direction. In the treatise by Miss DIEHL, Miss GORTER, VAN ITERSON and Miss KLEINHOONTE instances are also cited from recent literature. From these observations we may conclude that in the growth of cell walls new cellulose micellae are deposited preferably laterally to the micellae present. This also explains why the cell arms of the pith cells once they have been formed, grow especially in the direction of those arms. This will be the case even when the surrounding tissue has ceased to stretch those arms. Meanwhile it is by no means impossible that this stretching is continued and it may be that it has a conducive effect on the longitudinal growth of the arms; we would remark here that in consequence of the preferential direction of the micellae the arms are more extensible in longitudinal than in transverse direction. It is possible that this extension creates room for new micellae; the new-deposited micellae will also have a preference for the transverse direction on account of the unequal stretching in the various directions of the wall of the cell arms.

The stronger double refraction of the longer cell arms described above under 3 is probably to be ascribed to a perfectioning process of the "Röhrenstruktur", attended with the longitudinal growth.

Finally we think we can make a suggestion as to the cause of the fact that in the layers formed in the arms at a later stage the micellae are deposited in longitudinal direction and no longer transversely. We mention here that in thickened cell walls the micellae in layers deposited later generally prefer a direction different from that in the initial wall.

We will now point out that through strong extension of a wall in a certain direction the extensibility in that direction becomes smaller and may finally be even very slight (see in this connection the observations about elastic and plastic deformations on pp. 793—796 in the treatise by Miss DIEHL, Miss GORTER, VAN ITERSON and Miss KLEINHOONTE), whereas the extensibility in the direction perpendicular to it is maintained and may even become greater. It is conceivable that before the extra thickening in the cell arms occurs, the extensibility of the wall in the transverse direction has become so slight that the directing action of the turgor becomes inactive in transverse direction on the new-deposited micellae. Under these circumstances the turgor or the mechanical stretching of the surrounding tissue might exclusively call forth stretching in longitudinal direction of the cell arms. The micellae forming the new layers might be directed under the influence of this stretching and hence have a preference for the longitudinal direction of the cell arms.

5. *Summary.*

On the ground of the considerations expressed in the foregoing pages we think we may point out the following circumstances as causing the stellate form of the pith cells of *Juncus*:

a. the special structure of the meristematic tissue, from which those cells are formed, which tissue is namely to be considered as a complex of vertical rows of cubo-octahedrons, piled up on hexagonal faces,

b. the tendency of the polyhedral cells at a very juvenile stage to rounding off near the vertices in consequence of the turgor pressure and their tendency to remain flattened in the centre of the attached polyhedral faces,

c. at a slightly older stage: the mechanical stretching of the pith in radial and to a less extent in vertical directions through the growth of the surrounding tissue, owing to which the intercellular spaces originally bounded by concave faces, are changed into spaces bounded by convex faces, which causes the first stage in the development of the cell arms,

d. the turgor pressure in the cylindrical cell arms through which the walls of those arms are stretched more in transverse than in longitudinal direction, so that the micellae in those walls obtain a preference for a transverse direction, while this enables the arms to grow in length, although

perhaps by the side of this, in this stage too, the mechanical stretching mentioned sub *c* has a conducive effect on the longitudinal growth,

e. at the last stage: too slight an extensibility of the cell arms in the transverse direction to expect a directing effect on the new-deposited micellae, but now the occurrence of a directing influence of the turgor pressure or of the mechanical tension mentioned sub *c* in longitudinal direction of the cell arms on these new micellae, which are deposited as a layer with a preference of the micellae for the direction of the cell arms.

This investigation was made under the direction of Prof. G. VAN ITERSON Jr. in the laboratory for technical botany of the "University College of Technology" at Delft.